2-Methoxyestradiol : A 17 -Estradiol Metabolite With Gender-Independent Therapeutic Potential

Dubey, Raghvendra K

DOI: https://doi.org/10.1161/HYPERTENSIONAHA.117.09265

2-Methoxyestradiol

A 17β-Estradiol Metabolite With Gender-Independent Therapeutic Potential

Raghvendra K. Dubey

See related article, pp 1104–1112

Observational studies in humans and experimental studies in animals provide evidence that estrogens protect postmenopausal women against cardiovascular disease.[1] However, results from randomized clinical trials do not support this contention.[1] The reason(s) for the disparity in the outcome remains unclear and unresolved. Multiple factors, including type of estrogen used, interaction with progesterone, age of subjects, the status of vascular disease, and the timing of treatment, have been hypothesized to contribute to lack of protective actions in the clinical trials.[1,2]

On the basis of the conventional mechanism, major focus has been directed toward the role of estrogen receptors in mediating cardiovascular effects of estrogen, and much less attention placed on the contribution, consequence, and importance of estrogen metabolism in defining the protective actions of estrogen, particularly, 17β-estradiol. In this issue Pingili et al[3] demonstrate that 2-methoxyestradiol, an endogenous 17β-estradiol metabolite, attenuates angiotensin II–induced hypertension and renal dysfunction in intact male and ovariectomized female CYP1B1$^{+/+}$ mice.[3] Previously, using CYP1B1$^{-/-}$ mice and pharmacological CYP1B1 inhibitor, their team demonstrated that conversion of endogenous 17β-estradiol to 2-methoxyestradiol is essential for counteracting angiotensin II–induced hypertension and end-organ damage (renal and cardiovascular) in female mice.[4] Collectively, their findings provide credence to the original hypothesis that 2-methoxyestradiol mediates the protective actions of 17β-estradiol on cardiovascular and renal system.[2]

Aromatase metabolizes testosterone to 17β-estradiol, whereas multiple CYP450-isoforms (CYP1A1, CYP1A2, CYP1B1, and CYP3A4) convert 17β-estradiol to several metabolites.[5] The CYP1B1 isozyme is largely extrahepatic and dynamically expressed in the vascular endothelial and smooth muscle cells. It metabolizes 17β-estradiol to 2- and 4-hydroxyestradiol, the precursors for 2- and 4-methoxyestradiol.[5] Oxidative metabolism by CYP1B1 generates free radicals and associated with pulmonary hypertension, atherosclerosis, renal disease, and glaucoma.[2,5–7] Interestingly, sequential conversion of estradiol to 2-hydroxyestradiol and 2-methoxyestradiol by CYP1B1 and catechol-O-methyltransferase (COMT), respectively, protects females against cardiovascular disease and responsible for sex-based differences.[2,4,6] In contrast to 2-methylation, further oxidation of 17β-estradiol and estrone metabolites such as 2/4-hydroxyestradiol, 2/4-hydroxyestrone, 16α-hydroxyestrone, and 16α-hydroxyestradiol can lead to formation of highly reactive quinones and semiquinones, which are mutagenic and proproliferative and implicated in hypertension and vascular remodeling associated with occlusion in pulmonary hypertension.[5–7] Importantly, 2-methoxyestradiol blocks semiquinone/quinone formation by inhibiting CYP1B1 via concentration-dependent feedback mechanism.[2] Hence, ying-yang balance between protective (2-methoxyestradiol) and deleterious (16α-hydroxyestrone or 4-hydroxyestradiol) 17β-estradiol metabolites may play a critical role in defining its protective actions on the cardiovascular system (Figure).

CYP1B1 not only metabolizes 17β-estradiol, but also converts testosterone to 6β-hydroxytestosterone and 16α-hydroxytestosterone.[3] The effects of angiotensin II–induced hypertension and end-organ damage in CYP1B1$^{+/+}$, but not CYP1B1$^{-/-}$ male mice, were mediated by 6β-hydroxytestosterone.[3] In females, conversion of endogenous 17β-estradiol to 2-methoxyestradiol, but not 4-methoxyestradiol (a 4-hydroxyestradiol product), protected against angiotensin II–induced hypertension and end-organ damage.[4] Here, they provide conclusive evidence that 2-methoxyestradiol reduces angiotensin II–induced hypertension and prevents renal dysfunction in intact males and ovariectomized female mice.[3] Interestingly, circulating levels of 2-methoxyestradiol are lower in subjects with preeclampsia and inversely correlate with hypertension.[8] Moreover, 2-methoxyestradiol lowers blood pressure induced by deoxycorticosterone acetate salt and attenuates hypertension in spontaneously hypertensive and obese ZSF1 rats and in animal models for pulmonary hypertension and renal toxicity.[2,3] The antihypertensive effects of 2-methoxyestradiol were accompanied with improvement in endothelial function and inhibition of vascular, cardiac, renal, and pulmonary remodeling associated with the cell proliferation in the respective diseases.[2,3] Hence, exogenous administration of 2-methoxyestradiol is capable of overriding the deleterious actions of other hydroxyestrogens and quinone/semiquinones,

From the Department of Reproductive Endocrinology, University Hospital Zurich, Switzerland; Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland; and Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, PA.

Correspondence to Raghvendra K. Dubey, Department for Reproductive Endocrinology, University Hospital Zurich, Wagistrasse 14, CH-8952 Schlieren, 8091 Zurich, Switzerland. E-mail raghvendra.dubey@usz.ch

(*Hypertension*. 2017;69:1014-1016.
DOI: 10.1161/HYPERTENSIONAHA.117.09265.)

Hypertension is available at http://hyper.ahajournals.org
DOI: 10.1161/HYPERTENSIONAHA.117.09265

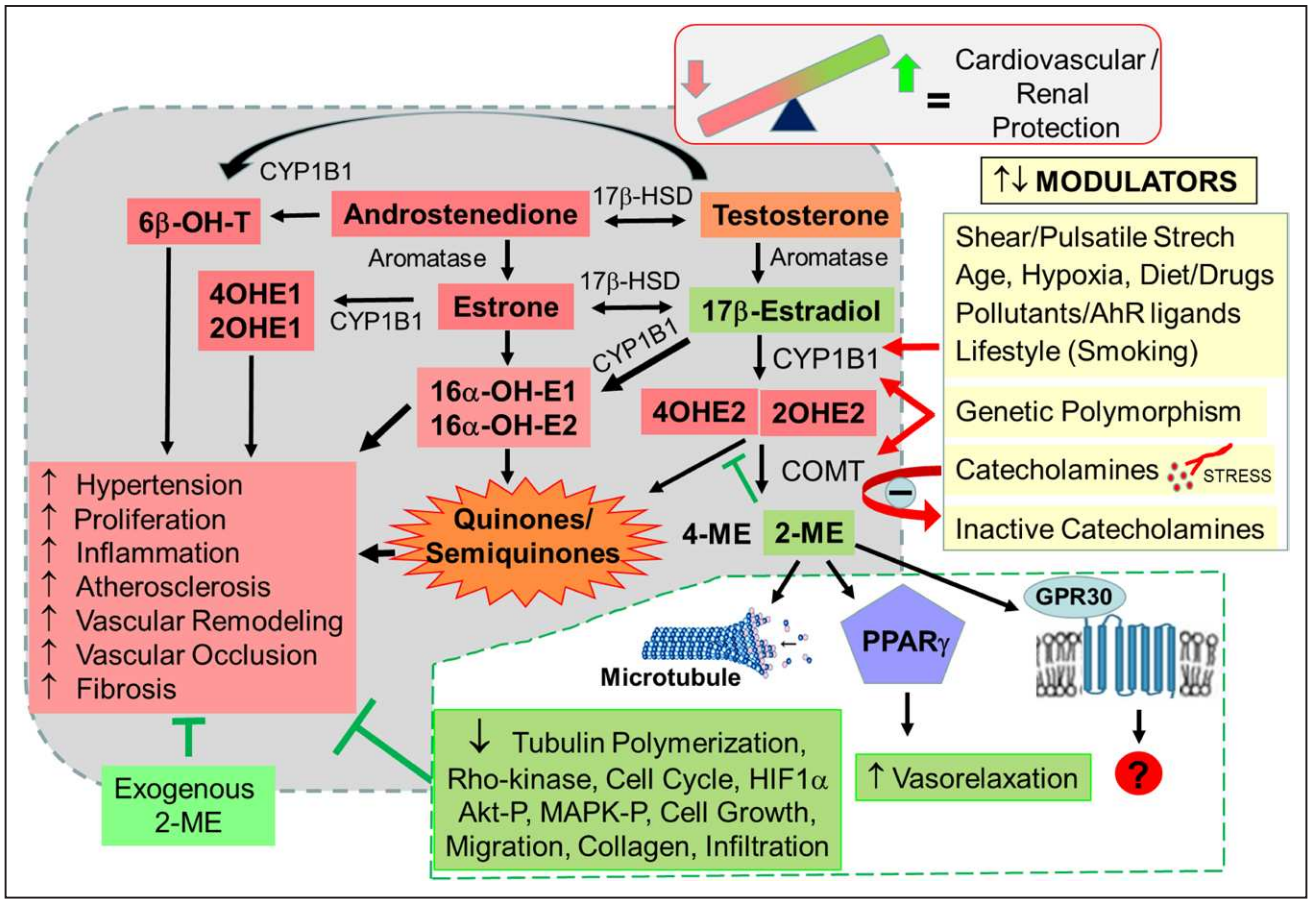

Figure. The ying-yang balance between deleterious and beneficial 17β-estradiol and testosterone metabolites generated by cytochrome-P1B1 (CYP1B1) and catechol-O-methyltransferase (COMT) in the vasculature. Exogenous 2-methoxyestradiol (2-ME) overrides the deleterious actions of endogenous metabolites of testosterone[2] and estrogen(s) on hypertension and cardiovascular/renal dysfunction.[3] Moreover, it depicts the conversion of 2-hydroxyestradiol (2OHE2) to 2-ME by COMT, the factors modulating CYP1B1 and COMT activity, and the multiple protective effects 2-ME induces and the potential receptors involved. ⊥ indicates inhibition; ↑ increase; ↓ decrease; and ↔ bidirectional. 17β-HSD indicates 17β-hydroxysteroid dehydrogenase; 6β-OH-T, 6β-hydroxytestosterone; AhR, arylhydrocarbon receptor; E1, estrone; and E2, estradiol.

implicated in the pathophysiology of estrogen-induced pulmonary hypertension (Figure).[7]

The metabolism of endogenous 17β-estradiol to beneficial or deleterious metabolites may explain the lack of protection by estrogen therapy in randomized clinical trials. First, use of Premarin, which contains mare urine-derived estrogens has negligible amounts of 17β-estradiol, the major endogenous human estrogen and 2-methoxyestradiol precursor.[6] Second, CYP1B1 activity can be influenced by multiple modulators (age, hypoxia, mechanical shear and pulsatile stress, dietary factors, endocrine disruptors and endogenous phenols, pollutants, lifestyle, eg, drugs and smoking, and genetic polymorphism) which may dysbalance the formation of both 2-methoxyestradiol and the deleterious 17β-estradiol metabolites (Figure).[5] Hence, the positive outcome in animals given 17β-estradiol and housed under controlled conditions would be difficult to mimic in humans. Third, the conversion of 2-hydroxyestradiol to 2-methoxyestradiol by COMT can be inhibited by genetic polymorphism and catecholamines.[2,5]

Although, role of 17β-estradiol–derived 2-methoxyestradiol in preventing cardiovascular disease is debated,[9] exogenous administration of 2-methoxyestradiol clearly prevents multiple proliferative disorders, including vascular occlusion, atherosclerosis, pulmonary hypertension, systemic sclerosis, and cancer.[2,3] Hence, 2-methoxyestradiol, in itself, could be an important therapeutic agent against proliferative diseases. Risk of cancer limits the use of estrogen therapy in postmenopausal women. Findings that 2-hydroxylation is protective against breast cancer and that 2-methoxyestradiol is anticarcinogenic suggest that it may be a safer, noncarcinogenic, substitute for treating cardiovascular disease in postmenopausal women.[2,5]

The intracellular mechanism(s) via which 2-methoxyestradiol inhibits cell growth, cell migration, and infiltration of macrophages/monocytes and induces apoptosis and anti-inflammatory effects are well documented.[2] However, which receptor(s) mediate its effect(s) remains unknown. Interaction of 2-methoxyestradiol with colchicine binding site inhibits tubulin polymerization, which plays a key role in cell division and motility.[2] Reportedly, 2-methoxyestradiol binds to GPR30 (G-Protein–Coupled Estrogen Receptor-30); however, no role for GPR30 was found in functional studies.[3] Structural similarity of 2-methoxyestradiol to rosiglitazone suggests that it may be a PPARγ (peroxisome proliferator-activated receptor gamma) ligand.[10] Interestingly, 2-methoxyestradiol–induced

endothelium-dependent relaxation and phosphorylation of Akt (protein kinase B) and eNOS (endothelial constitutive nitric oxide synthase) are abrogated when PPARγ was blocked or silenced.[10] Further examination of 2-methoxyestradiol binding sites using sensitive analytic approaches such as proteomics would be of considerable value.

On the basis of current data, 2-methoxyestradiol cannot be considered a substitute for estrogen therapy. For example, the effectiveness of 2-methoxyestradiol against postmenopausal symptoms, such as hot flushes, night sweats, osteoporosis, and urogenital dryness, and whether it is devoid of prothrombotic effects, remains unknown. However, based on its beneficial actions, combining 2-methoxyestradiol with existing estrogen replacement therapies may potentially increase their benefits.

Because 2-methoxyestradiol is nonfeminizing, the possibility that it can override the deleterious actions of CYP1B1-derived metabolites of androgens and estrogens on hypertension and cardiovascular and renal system suggests that it could be of therapeutic importance in both women and men. The major challenge in developing 2-methoxyestradiol as a useful drug is overcoming its undesirable pharmacokinetic properties, that is, poor oral bioavailability and short half-life. To develop more potent 2-methoxyestradiol analogs, we need to identify its functional receptor and transporters.

Acknowledgments

Because of limitations of words, many original articles were not cited.

Sources of Funding

This work was supported by grants from Swiss National Science Foundation Grants: IZERZO-142213 and 31003A-138067, and UNISCIENTIA Stiftung Nr. P100-2016.

Disclosures

R.K. Dubey is an inventor on US patent number-6998395.

References

1. Alhurani RE, Chahal CA, Ahmed AT, Mohamed EA, Miller VM. Sex hormone therapy and progression of cardiovascular disease in menopausal women. *Clin Sci*. 2016;130:1065–1074. doi: 10.1042/CS20160042.
2. Dubey RK, Jackson EK. Potential vascular actions of 2-methoxyestradiol. *Trends Endocrinol Metab*. 2009;20:374–379. doi: 10.1016/j.tem.2009.04.007.
3. Pingili AK, Davidge KN, Thirunavukkarasu S, Khan NS, Katsurada A, Majid DSA, Gonzalez FJ, Navar LG, Malik KU. 2-Methoxyestradiol reduces angiotensin II–induced hypertension and renal dysfunction in ovariectomized female and intact male mice. *Hypertension*. 2017;69:1104–1112. doi: 10.1161/HYPERTENSIONAHA.117.09175.
4. Jennings BL, George LW, Pingili AK, Khan NS, Estes AM, Fang XR, Gonzalez FJ, Malik KU. Estrogen metabolism by cytochrome P450 1B1 modulates the hypertensive effect of angiotensin II in female mice. *Hypertension*. 2014;64:134–140. doi: 10.1161/HYPERTENSIONAHA.114.03275.
5. Zhu BT, Conney AH. Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis*. 1998;19:1–27.
6. Lindsey SH. Importance of estrogen metabolites. *Hypertension*. 2014;64:21–22. doi: 10.1161/HYPERTENSIONAHA.114.03382.
7. Hood KY, Montezano AC, Harvey AP, Nilsen M, MacLean MR, Touyz RM. Nicotinamide adenine dinucleotide phosphate oxidase-mediated redox signaling and vascular remodeling by 16α-hydroxyestrone in human pulmonary artery cells: implications in pulmonary arterial hypertension. *Hypertension*. 2016;68:796–808. doi: 10.1161/HYPERTENSIONAHA.116.07668.
8. Pertegal M, Fenoy FJ, Bonacasa B, Mendiola J, Delgado JL, Hernández M, Salom MG, Bosch V, Hernández I. 2-methoxyestradiol plasma levels are associated with clinical severity indices and biomarkers of preeclampsia. *Reprod Sci*. 2015;22:198–206. doi: 10.1177/1933719114537716.
9. Wilhelmson AS, Bourghardt-Fagman J, Gogos JA, Fogelstrand P, Tivesten A. Catechol-O-methyltransferase is dispensable for vascular protection by estradiol in mouse models of atherosclerosis and neointima formation. *Endocrinology*. 2011;152:4683–4690. doi: 10.1210/en.2011-1458.
10. Chen W, Cui Y, Zheng S, Huang J, Li P, Simoncini T, Zhang Y, Fu X. 2-Methoxyestradiol induces vasodilation by stimulating NO release via PPARγ/PI3K/Akt Pathway. *PLoS One*. 2015;10:e0118902. doi: 10.1371/journal.pone.0118902.

Hypertension
Journal of the American Heart Association

2-Methoxyestradiol: A 17β-Estradiol Metabolite With Gender-Independent Therapeutic Potential

Raghvendra K. Dubey

Hypertension. 2017;69:1014-1016; originally published online April 17, 2017;
doi: 10.1161/HYPERTENSIONAHA.117.09265
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563